BG-2019-198 Response To Reviews

Dear Professor Treude

Thank you for handling our manuscript, and for inviting a revised version. Please see below responses to all reviewer comments, followed by a marked up revised manuscript.

We thank the three reviewers for supportive and constructive comments. Reviewer comments are summarised / provided below, with replies and details of revisions in **bold**.

Yours sincerely,

Clare Woulds

Reviewer Comment 1

We thank the reviewer for their comment that the experiments we report are novel and elegant, and that our results are exciting. The reviewer raised the following substantive points:

The reviewer raises a valid question as to whether it is appropriate to scale up from processes measured in 10cm diameter cores to rates and measurements normalised to per m^2. **We acknowledge that presenting results as per cm^2 is a more conservative approach, however we note that per m^2 is the standard normalisation in the rest of the literature. We are happy to provide results normalised to cm^2, but feel strongly that the per m^2 version should also be presented to allow readers easy comparison with the wider literature. We leave this at the discretion of the editor.**

The reviewer notes that it does not make sense to report results as a mean and standard deviation when n=2. **We acknowledge that greater replication is certainly desirable, but not always achievable. Indeed, in this case further replication was prevented by the availability of cores, incubation equipment, and tha available time at sea. Results are already listed separately for A and B replicate cores in Table 2. Figures 2 and 3 have been re-plotted to avoid the use of mean and standard deviation values, and now match the style of Fig. 5. Mean values have been replaced with ranges in the text describing the experimental results.**

In addition the revierew requested the following minor changes:

Page 1 Line 1: Should read “Benthic carbon fixation….” **corrected**

Page 1 Line 14-15: “There are no previous direct…” This sentence is not required. Please
delete the sentence. **We would prefer to keep this sentence to highlight the novelty of our study, and leave this at the discretion of the editor.**

Style Definition: Heading 1: Font: 17 pt, Space Before: 0 pt, After: 10.5 pt, Pattern: Clear (White)

Style Definition: Heading 3: Outline numbered + Level: 2 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0.63 cm + Indent at: 1.27

Page 1 Lines 15-16: Remove paragraph break. **Removed**
Page 1 Lines 21-22: Remove paragraph break. **Removed**
Page 1 Line 22: Revise to: 'Fixation of inorganic C into bacterial biomass was observed in all cores/sites.' Please revise as suggested. **Added 'sites' to correct**
Page 3 Line 30 – Page 4 Line 85: Throughout the introduction there are many uses of 'therefore' and 'however'. 90 % of the time these words are superfluous. Please revise the introduction to make less use of them. **Text revised to reduce incidence of 'however' and 'therefore'.**
Page 3 Line 32. Split this into two sentences. '…dissolved sulphides and methane. This supports microbes that combine…' **Changed**
Page 4 Line 63-64: Do you have any supporting literature that can be cited to support this sentence. **The reference supporting this statement (Bernardino et al., 2012) is provided at the end of the following sentence, once the point is fully made.**
Page 4 Line 72: 'On the contrary however' please revise, this is not well phrased. **Deleted 'however'**
Page 4 Line 77: Delete sub-heading **Deleted**
Page 4 Line 80: Hypotheses should be 'tested' not 'addressed' **Corrected**
Page 5 Line 108-119: This provides a brief summary of the experimental methods. Please refer to an alternative source as (following…) where a more detailed description of the method can be found. **The method is not published at greater length elsewhere, and all details have been provided. We are happy to add further details that are requested (such as those relating to C dose which have been added in response to other reviews).**
Page 5 Line 111-112: *Chlorella spp.* phytodetritus would not be representative of the algal material processed in Antarctic systems. A diatom would have been a more appropriate choice of 13C-labelled substrate. **We acknowledge this point, and have added text to the method section, as detailed in response to a similar point made by reviewer 3 (see below).**
Page 5 Lines 119-119: Half a core seems to be a very small volume of sediment for conducting macrobenthic analysis. Given that the size range of macrobenthic fauna is variable, and species are mobile, is this sample volume appropriate? I get the impression that you may be missing something significant by only focusing on half a core for the bacterial and macrobenthic communities. **We acknowledge this point. This is a standard limitation for isotope tracing experiments, from which samples are needed for a range of different analyses. In addition, when conducted in the deep sea, cores tend to be of relatively small diameter (as opposed to 14-25 cm diameter cores which can be used in shallower settings). We stress that we do not attempt to present a macrofaunal survey based on the organisms picked from our experimental cores, as the volume of sediment used would certainly be too small for that purpose.**
Page 6 Lines 151-158: A lot of potential data has been discarded from the PLFAs by just focusing on four 'bacteria-specific' fatty acids. It would be interesting to see the full profiles, particularly as the 13C-labelled bicarbonate treatment may reveal some insight into which PLFAs might be good indicators of microbial carbon fixation. **The PLFA suites are presented in Figure 4, and described and interpreted in section 3.2. We considered the use of PLFA suites carefully during manuscript preparation, and are not confident in drawing further conclusions from them.**

As previously mentioned, I am not content with the use of standard deviations to describe variation in the data. Where n = 2, you cannot reliably calculate means or standard deviations. **Figures and text have been amended accordingly.**

Page 7 Line 168: 'In *the* algae addition experiments…' Please revise. **Corrected**

Page 8 Lines 174-181: I think you could potentially offer more insight into the microbial processes by considering a wider range of PLFAs for each site. Which PLFA groups showed greatest label uptake? **We appreciate this suggestion. However, the scope to further interrogate the PLFA data has already been carefully considered. We decided that further conclusions cannot be drawn with an acceptable level of confidence.**

Page 8 Line 175: Normally C19:0 is used as a standard in the PLFA analysis which may explain why it is found in higher concentrations. **Apologies, C19:0 was indeed used as a standard The values in the text and figures have been adjusted to exclude it.**

Page 8 Line 183: Please revise to 'Faunal uptake of added C differed between the two replicate cores in all experiments…'**Revised**

Page 8 Line 191-192 and Figure 6: Given the small sample size, I am not convinced that a community level analysis of faunal feeding responses is appropriate. Differences in faunal uptake are likely to be driven by spatial variability, with common taxa such as polychaetes heavily overrepresented. This leads to the 'mixed macrofauna' category essentially consisting of everything except polychaetes. **We agree that the taxonomic resolution of the data is low, but we still feel it is worth reporting the available information on the identities of the organisms responsible for C uptake. We therefore chose to keep this short section, but have prefaced it with the following text to ensure that the limitation is acknowledged:** 'Small size of individuals meant that organisms had to be pooled for isotopic analysis, limiting the taxonomic resolution of the faunal uptake data. Although limited in this way, the data show that […]'

Page 8 Line 191 – Page 9 Line 199: In light of the small sample size please don't refer to dominance either in terms of faunal abundance or feeding responses. It would be more appropriate to discuss simply which groups were more/less abundant and exhibited greater/weaker uptake of the 13C-label. **This section has been edited to avoid using the term 'dominant' or 'dominance'.**

Page 8 Line 196- Page 9 Line 199: This last sentence is confusing, please revise and clarify. **The sentence has been revised to**: 'In addition, meiofaunal organisms took up ^{13}C at Middle Sister, and the bicarbonate ^{13}C that was transferred to macrofauna at Hook Ridge was mostly observed in amphipod crustaceans.'

There is frequent use of 'therefore' and 'however', please remove these where possible. **Discussion text has been edited to remove several uses of each.**

Page 9 Lines 202-212: This paragraph is a description of the results. Please revise to contextualize your findings. **This text has been edited slightly, but we feel that these features of the data need to be pointed out as a foundation for the material that follows.**

Page 9 Lines 220-222: Long sentence, requires broken up. Please revise. **This sentence has been edited, and broken into two.**

Page 9 Lines 223 Page 10 Line 228: Please revise along the lines of "This is supported by a recent modelling study which suggested that…. (Bell et al 2017b). Similar results have also been reported from the methane-rich non-hydrothermal sediments… (Woulds et al., inpress). **The text has been shortened along the lines suggested.**

Page 10 Line 242-248: I am afraid that this is a major flaw in the overall paper. Given that temperature is critical to microbial metabolism, the current paper is likely to seriously underestimate the level of carbon fixation. This needs to be made clearer earlier in the paper. **The extent to which this is a problem remains an open question. Observations of cores on deck strongly suggested that the in situ temperature was substantially lower than that calculated (by estimating cooling of cores during recovery) by Klinkhammer et al. (2001) – i.e. the extent of hydrothermal venting could have changed over time. Unfortunately, due to kit malfunction, we did not have equipment available for in situ sediment temperature measurements. In the absence of this we feel that the experimental approach used here has as much chance of being correct as if we had used the in situ temperature calculated by Klinkhammer et al several years earlier. Considering this, we feel that acknowledgement and discussion of the potential impact of temperature is correctly placed here.**

Page 11 Lines 273-275: Based on two replicates, it would only be possible to discuss the magnitude of the differences and perhaps compare these between sites. Remove reference to standard deviations from the discussion. **Reference to standard deviation has been removed.**

Page 12 Line 285: Delete 'rather' **Deleted**

Page 12 Line 298: Revise to '…Branfield Strait. Therefore…' **A slightly different edit has been made in response to an earlier comment.**

Page 12 Lines 298-299: Here you are discussing the effects of temperature on metabolic rates. Here you should consider the impacts of rate limitation and do a quick literature search. There is quite a large body of literature on this topic **It is not entirely clear what point the reviewer would like to see added. However, we have done the suggested search, and have added the following:** 'Both low temperature and food scarcity have previously been observed to limit metabolic rates in polar environments (Brockington and Peck, 2001; Sommer and Portner, 2002).'

Page 12 Line 302: Delete 'Thus' **Deleted**

Page 13 Line 324-325: Based on the Q10 effect, metabolic activity increases logarithmically with temperature. As such, a change of 1oC may be more significant than you assume. I think this may require further explanation. **We acknowledge this theoretical point. However, previous studies which have examined the impact of temperature on this type of experiment (Moodley et al., 2005; Woulds et al., 2009) have not found an exponential response, so extensive additional discussion may not be well founded. We have edited text to allow for the fact that a 1 degree temperature difference could have accounted for part of the difference.**

Page 13 Line 339: Delete 'and thus high biomass benthic communities.' **Deleted**

Page 13 Line 341: Delete 'Further' and replace 'while' with 'Whilst' **Revised**

Page 13 Line 341-342: The comparison between sites was limited by the size of each sample (half a core), and lack of replication (n = 2). Your experimental design does not allow you to make any inferences on faunal patchiness. **We accept this limitation, but have noted earlier that a greater degree of variability between replicates was observed then in other experiments conducted in the same way. We feel that it would be remiss to remove mention of faunal patchiness.**

Page 14 Line 347: You cannot use the term 'significant' as this implies the use of inferential statistical tests. Please revise. **Apologies. The text has been edited to avoid the use of 'significant' here and elsewhere.**

Page 14 Line 354: Replace 'dominant' with 'main' **Revised**

Page 14 Line 357-358: 'Therefore the hydrothermal site (Hook Ridge) in this study was not the hotspot of C-cycling that we hypothothesised it would be.' You need to define what is meant here by a 'hotspot of C-cycling.' Is this referring to chemosynthetic carbon fixation? **This has been clarified, and now reads '**The hydrothermal site (Hook Ridge) in this study did not show more rapid C-cycling than other similar experiments, as we hypothesised it would.'

Page 14 Line 358-359: Delete paragraph break. **We would prefer to keep two separate paragraphs, one for each of the main topics of our manuscript.**

Page 14 Line 362: Delete the final sentence. **We would prefer to keep this final statement as a pointer towards future work.**

Reviewer Comment 2

Reviewer 2 argues that the measurements of in situ C fixation that we present have conceptual flaws, due to having been conducted ex-situ. Thus the sediment that we incubated was cut off from its supply of the electron donors which provide the energy for chemosynthesis, and which are presumably sourced from the upwards flux of hydrothermal fluid from deeper in the sediment. **We acknowledge this point, but suggest that it may not have been a serious consideration at the sites which we studied, and does not warrant exclusion of all the benthic inorganic C fixation material.**

Firstly, the hydrothermal site that we studied (Hook Ridge, in the Bransfield Strait) was rather mildly hydrothermal. Hence, as has been reported, vent endemic fauna were almost absent (Bell et al., 2016), there was no increase in faunal biomass close to venting, and downcore profiles of alkalinity, nitrate and ammonium were consistent with normal microbial processes (Aquilina et al., 2013). There were indications of of hydrothermal flux in chloride, sulphate and sulphide profiles, which allowed Aquilina et al. (2013) to calculate hydrothermal advection rates of 9-33 cm y^{-1}. At these low advection rates we suggest that there would not have been sufficient time during our ~60 h experiment for a noticeable depletion in availability of electron donors supplied by hydrothermal fluid.

Secondly, we measured greatest amounts of benthic inorganic C fixation at our non-hydrothermal control site. The methods we used did not allow us to definitively pinpoint the metabolic processes responsible for inorganic C fixation, but the fact that C fixation was maximal at a non-hydrothermal site suggests that it is not, or not always, inherently linked to hydrothermalism. Indeed this is one of our key findings. Therefore, while our ex-situ incubation

technique could have resulted in conservative rate measurements at the hydrothermal site, we do not feel that it would be a proportionate response to exclude all the material about benthic inorganic C fixation.

We have added the following to discussion section 4.1:

'Experiments were designed to replicate natural conditions as far as practically possible, while being limited to shipboard rather than in situ methods. One result of this is that the sediment contained in cores was detached from the upward flux of hydrothermal fluid, and the electron donors it supplied. This could have limited inorganic C fixation, which would have impacted the rates measured at Hook Ridge. We suggest however that this is not a serious limitation, as Hook Ridge was rather mildly hydrothermal. Vent endemic fauna were almost absent (Bell et al., 2016), there was no increase in faunal biomass close to venting, downcore profiles of alkalinity, nitrate and ammonium were consistent with normal microbial processes, and hydrothermal advection rates were 9-33 cm y^{-1} (Aquilina et al., 2013). At these low advection rates we suggest that there would not have been sufficient time during our ~60 h experiments for a noticeable depletion in availability of electron donors supplied by hydrothermal fluid.'

In addition the reviewer asks for clarification of methods (e.g depths over which PLFAs were measured, and procedure used to determine whether labelling levels were above background). These details will all be added. Further they also make the same point about use of means and standard deviations as reviewer 1. As stated in the reply to reviewer 1, we will alter our presentation of results to avoid use of means and standard deviations.

References:

Aquilina, A., Connelly, D. P., Copley, J. T., Green, D. R. H., Hawkes, J. A., Hepburn, L. E., Huvenne, V. A. I., Marsh, L., Mills, R. A., and Tyler, P. A.: Geochemical and Visual Indicators of Hydrothermal Fluid Flow through a Sediment-Hosted Volcanic Ridge in the Central Bransfield Basin (Antarctica), Plos One, 8, 2013

Bell, J. B., Woulds, C., Brown, L. E., Sweeting, C. J., Reid, W. D. K., Little, C. T. S., and Glover, A. G.: Macrofaunal ecology of sedimented hydrothermal vents in the Bransfield Strait, Antarctica, Frontiers in Marine Science, 3, 2016

Line 57 to 62 It is better to mention about the time scale, because C uptake by fauna will also be respired into CO2 in longer time scale. **'In the short term' added to line 54.**

Line 93 Middle sister is not described in Fig 1 (Three Sisters is described). Off-vent is also not described in Fig 1, but Off-Axis control is described. Please be consistent through the text and the figures. **Unfortunately re-drawing the figure is not straightforward, however the caption states that 'off-axis control' is the same as 'off vent', and 'Three Sisters' is the same as 'Middle Sister'.**

Table 1 The authors listed the water temperature of each site, but do you have a data for characterizing each site in terms of venting activities, such as heat flow value, H+ or CH4 or Cl concentration of pore water? Can you list up some from Aquilina et al. 2013?? **We do not have the parameters mentioned by the reviewer for all sites (due to low hydrothermal advection), so do not feel that it would be appropriate to add to Table 1. However, we have added the following note to the method section: '**Porewater geochemistry at Middle Sister and Off-Vent

were consistent with microbial processes without influence of hydrothermal activity. Porewater NO_3^- and NH_4^+ profiles were indicative of nitrate reduction, but downcore declines in SO_4^{2-} and Cl^- were lacking over the ~40 cm depth sampled. In contrast, at Hook Ridge SO_4^{2-} was depleted by up to 11% compared to seawater, and Cl^- by up to 7%, allowing calculation of hydrothermal advection of 9-33 cm y^{-1} (Aquilina et al., 2013).'

Line 115 (If the authors decided not to delete Chemoautotrophic C production results) To give better idea how much 13C- and 15N were dosed into existing DIC or ammonium, it is needed to indicate them uM. In the line 158, the authors mentioned that the added 13C-DIC account for 22%, but this must differ between sites because venting fluid contains high DIC (5-100mM) than bottom water (_2.1mM). **Estimated concentrations in porewaters of added substrates have been added. Due to weak hydrothermalism at Hook Ridge, alkalinity in the surface sediment there is similar to the other sites (although is higher further downcore, Aquilina et al., 2013), there fore there one estimated value is provided for all sites.**

Line 151 It is totally unclear which depths did authors use for each analysis. For PUFA, all sediment layers were used or not? For faunal, the authors examined 10 cm or deeper? **This detail has been added (0-1 cm for PLFAs, 0-10 cm for fauna).**

Line 173 More specifically, how much 13C-labeling was determined as detection limits considering natural variations in d13C values? This must be written in M&M. **M&M text details that natural isotopic baselines were used for individual PLFAs and faunal taxa. In addition, ^{13}C uptake was only calculated where the difference exceeded analytical variability. This detail has been added to M&M.**

Line 188 Again, how much 13C-enrichments were regarded as 13C uptake? "Measurable

uptake" sounds like even 1 per mil of d13C differences from background are

regarded as uptake. **See reply above.**

Line 206 Please describe this for more detail. Not only analytical precision, but also variations in background samples in replicate (if available) must be considered, which sometimes shows _5 per mil of variation. **Replicate background data are not available for PLFAs. For fauna they show variability of usually 1 per mil for each taxon, and enrichment was up to 68 per mil. We have been careful to use only data where we are confident there is an unambiguous and quantifiable enrichment (see earlier responses), and have applied further care in acknowledging the limitations of our study and not over-interpreting the data.**

Line 213 If you measured 13C of PLFAs in different layers, it is worth to put vertical trends in the graphs. **Due to resource constraints we only have these data for the surface 0-1 cm horizon, otherwise we would certainly plot the data downcore.**

Lines 250 and 252 Probably, "mg" is missing. **Corrected, thank you**

Line 273 It is odd that describing "standard deviation" on samples with 2 replicates. **In line with other reviewer comments we no longer use standard deviations.**

Figure 7 (If the authors decided not to delete Chemoautotrophic C production results)

It is a bit confusing to show these graphs together; one with “respiration” but one without. I understand that the respiration of B cannot be measured because of 13C-DIC addition, but you need to mention that clearly in the caption.

Line 325 The differences in microbial biomass were less than twice, while those in respiration rates differed _7 times. So, the microbial biomass is not the only reason. The authors need to discuss about these fact more carefully.

Reviewer Comment 3

We thank the reviewer for a supportive review.

The reviewer feels that the methods section could provide more detail, and this will be added in line with this and other reviews.

In particular, the reviewer asks for further detail of the dual labelled phytodetritus that was added to the ‘algae’ treatment, and this is provided. In addition acknowledge that the phytodetritus used was fresher and more reactive than the particulate organic matter that usually reaches the depth of our study sites. This is a common feature of most previous experiments of this type it means that the processing rates we report for algal are likely to be maximum rates. **We have added the following text to methods section 2.2:**

‘It is recognised that such organic detritus is less degraded than the sinking photosynthetic material which normally reaches the depths of our study sites. This is a limitation of the method common to all such experiments in the literature, and means that rates for processing of added C in ‘algae’ experiments should be considered maximal. Further, diatom detritus would have been more representative of local photosynthetic material, but was unfortunately not available.’

LN 83: “inorganic substrates” to bicarbonate (H13CO3-): **changed**

LN 103: A brief description here of the background macrofauna would be appropriate. **The following text has been added: ‘**. Polychaetes were numerically dominant (41-56%), except at Hook Ridge, which was dominated by peracarids, and oligochaetes were the next most dominant. Vent endemic fauna were represented by two species of siboglinid polychaete; S. *contortum* at Hook Ridge, and *Siboglimun sp*. Elsewhere (Bell et al., 2016a).’

LN 112: Product number for your labeled algal material is needed, CIL does not appear to sell a marine algal detritus that I could find by searching their site. Be careful with that description too, as it implies a bit of possible reworking given that you are working at 1000 m depth. If the material is the dual labeled lyophilized algal cells then it is really fresh algal material for application at a relatively deep site; you should discuss this if it is the case. An estimate of what portion of the annual flux this application represents would be appropriate to give more context to the amount of material be applied. **The part number has been added, as well as acknowledgement that the material is fresher than that which usually arrives at the site depth (see response to reviewer 2). The following has been added to provide context for the amount of algal C added ‘**This was equivalent to ~1.6% of total OC in the surface 1 cm of sediment, or ~9% of annual OC input (Bell et al., 2017b)’.

LN113: Context for the relative amount of application versus what is already there and available would be useful so the reader can gauge how large the applications are versus in situ backgrounds for C and N. **This has been added, please see above.**

LN 116: Describe the sampling intervals, this will help to indicate how many measurement points your rates are determined off of. **Detail added (sampling every 12 h for 60 h).**

LN 206: Provide a range of PLFA or organic ‰ 13C enrichments to support this claim. It will be more convincing to readers when presented in that manner. **Detail of the steps we took to ensure the reported uptake was real are given in the methods section. Since enrichments above background were often small, and baseline values themselves varied with taxon and specific PLFA, we feel that providing ranges here will be confusing rather than helpful. We will however provide access to archived data via a DOI, which we refer to in the results section.**

LN 212: I appreciate the candid nature of this statement, it shows a realistic interpretation of the data given the limited replication built into the study. **We thank the reviewer for this comment.**

LN 216: Provide a reference about the chemosynthetic endosymbionts, also an indication as to the nature of the symbionts, methane oxidizers or sulfur oxidizers would be appropriate. **References added, along with the detail that most of the endosymbionts will be doing sulphide oxidation.**

LN 235: “important aspect” Is this because it is minor, but potentially widespread? Vague as written. **This has been re-worded** ‘…chemoautotrophic C fixation may be considerably more widespread than previously thought. It is therefore deserving of further study so that it can be quantitatively incorporated into our understanding of the marine C-cycle.’

LN 244: So, this study likely represents minimum rates for chemosynthesis. The authors should phrase it that way and provide context of what the addition represented in comparison to normally available substrates. Better to focus on what your study has actually shown than to speculate that rates would have been higher if in situ temps were maintained. **We agree with the reviewer, the text has been re-phrased as ‘**It is therefore likely that the rates measured here for chemosynthetic incorporation of labelled bicarbonate are minimal rates.’

LN 250 & 252: 0.24-1.02 and 1.29 both need mg in front of C m-2 d-1, respectively **Corrected, thank you.**

LN 263: Would it be worth trying to isolate polar lipids from archaeal components given their slow metabolism and the relatively short time frame of this study? **This was initially an objective of the project. However, background organic geochemical work conducted by colleagues found archaeal lipids at very low concentrations, therefore we were very unlikely to succeed in tracing 13C into them given the volume of sample available.**

LN 268-277: Thank you for addressing the variability observed during tracer studies relying on bacterial mediation of a substrate! Is it worth talking about reasons for potential hotspots for both heterotrophy and chemosynthetic processes that are occurring in this system? I would expect variations in vent flows and sporadic

availability of resources to give rise to a community that readily adapts to changing conditions. **This is a good point. We have added the following statement to the relevant discussion section: '**Fine scale distribution of fauna has been show to relate to variations in concentrations of species such as sulphide and methane (Levin et al., 2003), therefore the patchiness observed especially at Hook Ridge is likely related to spatial and temporal fluctuation in hydrothermal advection.'.

LN 294: Provide percentages from the other studies here so the reader can directly compare these studies. **These have been added.**

LN 316: Does the time period involved in this incubation matter here? Transfer into symbiont and then into tube worm may take a bit more time and require a stronger signal to show up as the tracer is sequentially diluted through the two carbon pools? **Only fixation by endosymbionts would be required in order for labelled C to be detected in the isotopic signature of siboglinid specimens (no further transfer required, as the symbionts live within the annelid tissues). The rate of that process may have been a factor, and the following has been added along with other caveats:** '…or because experiments were not long enough for uptake by endosymbionts.'

Figures: Figure 1: state that depth is in meters in figure caption. **Added**

Figure 2 & 3: remove blue outline on bars. Considering the low uptake rates, consider converting into µg to limit the decimal places. But, you are consistent throughout currently. **Figures have been re-plotted in line with reviewer 1 comments, blue outlines have been removed. The reviewer makes a valid point about decimal places, but we prefer to use mg to maintain comparability with the literature.**

Figure 4: Format the letters for the figures into the actual graphs, hard to interpret as laid out presently. Also resulted in the splitting of the figure between page 24 and 25. Both substrates should be on the same y axis scale to aid in interpretation and comparison (both 60% max). **Letters not added to panels, but this can be done depending on what is preferred by typesetters. The reviewer makes a valid point about using the same y-axis scales, but this is not practical as it will make plots difficult to read – especially panel A (currently on 0-20% scale, so would be very small on a 0-60% scale).**

Benthic Carbon fixation and cycling in diffuse hydrothermal and background sediments in the Bransfield Strait, Antarctica

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Abstract

Sedimented hydrothermal vents are likely to be widespread compared to hard substrate hot vents. They host chemosynthetic microbial communities which fix inorganic C at the seafloor, as well as a wide range of macroinfauna, including vent-obligate and background non-vent taxa. There are no previous direct observations of Carbon cycling at a sedimented hydrothermal vent. We conducted ^{13}C isotope tracing experiments at 3 sedimented sites in the Bransfield Strait, Antarctica, which showed different degrees of hydrothermalism. Two experimental treatments were applied, with ^{13}C added as either algal detritus (photosynthetic C), or as bicarbonate (substrate for benthic C fixation). Algal ^{13}C was taken up by both bacteria and metazoan macrofaunal, but its dominant fate was respiration, as observed at deeper and more food limited sites elsewhere. Rates of ^{13}C uptake and respiration suggested that the diffuse hydrothermal site was not the hotspot of benthic C-cycling that we hypothesised it would be. Fixation of inorganic C into bacterial biomass was observed at all sites, and was measurable at 2 out of 3 sites. At all sites, newly fixed C was transferred to metazoan macrofauna. Fixation rates were relatively low compared to similar experiments elsewhere, thus C fixed at the seafloor was a minor C source for the benthic ecosystem. However, as the greatest amount of benthic C fixation occurred at the

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469 off vent (non-hydrothermal) site (0.077±0.034 mg C m^{-2} fixed during 60 h), we suggest that benthic fixation of
470 inorganic C is more widespread than previously thought, and warrants further study.
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1. Introduction

Sedimented hydrothermal vent (SHV) sites are those where hydrothermal fluid diffuses through soft sediment cover on its way to mixing with oceanic bottom water. This creates hot (up to ~100°C) sediments with porewaters rich in dissolved sulphide and methane. This supports microbes that conduct chemosynthetic C fixation through a range of pathways (Bernardino et al., 2012). These hydrothermally influenced sediments are likely to be more spatially extensive than hard substrate vents, although their diffusive nature makes their extent hard to quantify. Sedimented hydrothermal vents have been shown to influence biological community composition and nutrition at adjacent sites which were otherwise characterised as 'inactive' or 'off-vent' (Levin et al., 2009; Bell et al., 2016a; Bell et al. 2016b; Bell et al., 2017a). However, the ecology of sedimented hydrothermal sites has received relatively little study. There is only one modelling study that has focused on the interaction between benthic ecosystems and C-cycling at SHVs (Bell et al., 2017b), and there are no direct observations of SHV C-cycling by components of the benthic ecosystem.

So far, a limited number of studies have used natural stable isotopic analysis to determine carbon sources and their fixation pathways utilised by infauna at SHVs (Levin et al., 2009; Soto, 2009; Sweetman et al., 2013; Bell et al. 2016b; Portail et al. 2016). Evidence has shown that C fixed during anaerobic oxidation of methane, oxic methanotrophy, sulphide oxidation, as well photosynthetic organic matter (OM) sinking from the surface, are all utilised by macrofauna to varying extents at SHVs (Levin et al., 2009; Bernardino et al., 2012). It is challenging to quantify the relative contributions of different C sources to macrofaunal diets, both because the natural isotopic ranges of some C sources overlap, and because often the isotopic compositions of those end members could not be measured (Levin et al., 2009; Bell et al., 2016b). Unknown variability in trophic discrimination factors also currently preclude quantitative estimates of the relative contribution of different C sources.

Stable isotope tracing experiments offer a way to overcome some of these issues. The experimental addition of labelled C sources, either photosynthetic OM or dissolved inorganic C (bicarbonate) to SHV sediment allows production of chemosynthetic OM, and the transfer of different OM types into the macrobenthos and other C pools in the short term to be directly observed. Such experiments (using only photosynthetic OM) have been conducted at a wide range of (ostensibly) non-chemosynthetic benthic sites, and have shown a wide variation in the relative importance of different biological C processing pathways (Woulds et al., 2009; 2016). At food limited sites in the deep-sea, respiration tends to be the dominant fate of added OM (van Oevelen et al. 2011; 2012). Shallower, more food rich settings such as coastal fjords and estuaries, with greater sedimentary organic C concentrations and higher macrofaunal biomass, show a pattern of biological C processing in which uptake by

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fauna is a more important process, and at unusual and particularly food rich sites, such as the lower margin of the Arabian Sea oxygen minimum zone (~1000 m depth), macrofaunal C uptake can even be the dominant process (Woulds et al., 2009; 2016).

The occurrence of chemosynthesis in a benthic habitat represents an additional source of fresh, labile OM in an environment that would otherwise be more severely food limited. For this reason, it has been suggested that hydrothermally influenced sites can be biomass hotspots, where biogeochemical cycling is rapid (Bernardino et al., 2012). However, due to the environmental toxicity created by hydrothermal fluid, and the fact that the majority of taxa inhabiting SHVs are background rather than vent-endemic, the difference in faunal biomass between SHVs and adjacent non-vent sites is highly variable (Levin et al., 2009; Bernardino et al., 2012; Bell et al., 2016). It therefore seems possible that biological C processing at SHVs will show a distinct complement of biological C processing patterns unlike those observed elsewhere in the deep sea. The food rich, high biomass characteristics of some SHVs may lead to biological C processing that is and more similar to shallower, food rich environments. On the contrary, spatially variable biomass patterns, as well as the metabolic costs associated with potentially high temperatures and porewater toxicity could counteract the effect of enhanced food availability. As direct measurements of biological C processing rates and pathways have not previously been made at SHVs or in the Southern Ocean, there remains a gap in our understanding of sedimentary C and N-cycling.

In this study we conducted stable isotope tracing experiments at three sites of variable hydrothermal activity in the Bransfield Strait, Antarctica. To the best of our knowledge this is the first isotope tracing experiment in this type of system. The following hypotheses were tested:

- Hydrothermally influenced sites exhibiting chemosynthesis will show elevated rates of biological C processing.
- At hydrothermally influenced sites bicarbonate will be fixed by chemoautotrophs and transferred to the macrofauna.
- Preference for feeding on photosynthetic versus chemosynthetic OM will be taxon dependent.

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1.1 Hypotheses

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2. Methods

2.1 2.1 Study sites

In this study we focus on a SHV in the Bransfield Strait, close to the tip of the Antarctic peninsula. The discovery of hydrothermal venting in the Bransfield Strait was reported by Klinkhammer et al. (2001), who detected hydrothermal plumes in the water column, and recovered hot 'soupy' sediment from Hook Ridge. In addition, a species of *Sclerolinum* (Sahling et al., 2005; Georgieva et al., 2015) there has been described, and porewater geochemistry and hydrothermal flux rates have been published (Sahling et al., 2005; Aquilina et al., 2013).

Experiments were conducted at three sites in the Bransfield Strait, Antarctica (Fig. 1). Two of the sites lay on raised edifices, known as Hook Ridge and Middle Sister, along the axis of the basin, and were selected as being likely to exhibit diffuse hydrothermal venting, and the former was the location where diffuse venting had been identified. A third site, at a similar depth but along the north side of the basin, was chosen as an off-vent control (hereafter known as 'Off-Vent').

Porewater geochemistry at Middle Sister and Off-Vent were consistent with microbial processes without influence of hydrothermal activity. Porewater NO_3^- and NH_4^+ profiles were indicative of nitrate reduction, but downcore declines in SO_4^{2-} and Cl^- were lacking over the ~40 cm depth sampled. In contrast, at Hook Ridge SO_4^{2-} was depleted by up to 11% compared to seawater, and Cl^- by up to 7%, allowing calculation of hydrothermal advection of 9-33 cm y^{-1} (Aquilina et al., 2013).

Sediment organic carbon (Corg) concentrations were lower at Hook Ridge (0.97 wt% Corg) than at the Off-Vent and Middle Sister sites, which showed similar values (1.35 and 1.4 wt% Corg respectively, Table 1). The sites differed in biomass of different groups, with Hook Ridge and Middle Sister showing higher bacterial biomass and lower macrofaunal biomass than the Off-Vent site (Table 1). Hook Ridge was the only site classified as hydrothermally active by Aquilina et al. (2013). Porewaters were enriched in sulphide, methane and dissolved metals and depleted in chloride, and the calculated hydrothermal advection rate was 9-33 cm y^{-1}. Macrofauna tended to be representative of the background taxa of the region. Polychaetes were numerically dominant (41-56%), except at Hook Ridge, which was dominated by peracarids. Oligochaetes were the next most dominant at all sites. Vent endemic fauna were represented by two species of siboglinid polychaete; S. *contortum* at Hook Ridge, and *Siboglimun sp.* elsewhere (Bell et al., 2016a). Each site also supported one species of siboglinid

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574 polychaete. In the case of Hook Ridge this was *S. contortum*, and at Middle Sister and the Off-Vent site it was
575 *Siboglinum sp.*, and they were always a minority constituent of the community (Bell et al., 2016 a).

576 **2.2 Isotope tracing experiments**

577 Sediment cores (10 cm i.d.) were recovered using a multiple corer, and kept in the dark at seafloor temperatures
578 (Table 1) using cooled incubators. Experiments were initiated by addition of isotopically enriched substrates.
579 Cores were then sealed and incubated for ~60 h, during which core-top water was continuously stirred.

580 Duplicate cores were subjected to each of two treatments. In the 'algae' treatment, lyophilized algal cells
581 (*Chlorella*, Cambridge Isotope Laboratories, CNLM-455-1) enriched in ^{13}C and ^{15}N (both ~100 at %) were
582 allowed to settle on the sediment surface, giving a final dose of 436±30 mg C m^{-2}. This was equivalent to ~1.6%
583 of total OC in the surface 1 cm of sediment, or ~9% of annual OC input (Bell et al., 2017b). It is recognised that
584 such organic detritus is less degraded than the sinking photosynthetic material which normally reaches the
585 depths of our study sites. This is a limitation of the method common to all such experiments in the literature, and
586 means that rates for processing of added C in 'algae' experiments should be considered maximal. Further,
587 diatom detritus would have been more representative of local photosynthetic material, but was unfortunately not
588 available.

589 In the 'Bicarbonate' treatment a solution of 100 % ^{13}C labelled sodium bicarbonate and 100 % ^{15}N labelled
590 ammonium chloride was injected in the surface 5 cm of sediment porewater, to give a dose of 306 mg C m^{-2} and
591 2.52 mg N m^{-2}, and an estimated porewater bicarbonate concentration of 1 mM.

592 At intervals (T0 and every ~12 h thereafter) during the incubation, core top water samples were withdrawn from
593 Algae treatment cores, and stored in crimp-cap vials poisoned with $HgCl_2$ for dissolved inorganic carbon (DIC)
594 analysis. At the end of the experiment cores were extruded and sectioned at intervals of 0-1, 1-2, 2-3, 3-5 and 5-
595 10 cm. Half of each section was frozen at -20°C, and the other half was preserved in buffered 10% formalin.

596 **2.3 Sample processing and analysis**

597 Overlying water samples were analysed for concentration and isotopic composition of DIC in triplicate on a
598 Thermalox TOC analyser coupled to a Thermo Delta V Advantage IRMS via a Conflo IV interface, using a
599 Thermo TriPlus autosampler. The reaction column was filled with H_3PO_4-coated beads.

600 Frozen sediment samples were freeze dried, and surface 0-1 cm horizons were analysed for phospholipid fatty
601 acids (PLFAs) following Main et al. (2015). Briefly, samples were extracted in a modified Bligh and Dyer

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extraction solution of chloroform:methanol:citrate buffer, 1:2:0.8. The polar fraction was obtained by loading samples onto ISOLUTE SPE columns, washing with chloroform and acetone, and eluting with methanol. After addition of nonadecanoic acid (C19:0) as an internal standard, extracts were derivatised in the presence of KOH in methanol. Derivatisation was quenched with water and acetic acid, and the organic fraction was extracted by washing with 4:1 isohexane:chloroform. Samples were dried and then taken up in isohexane for analysis on a Trace Ultra GC, connected via a GC Combustion III to a Delta V Advantage IRMS (Thermo Finnigan, Bremen). The isotopic signature of each PLFA was measured against a CO_2 reference gas which is traceable to IAEA reference material NBS 19 TS-Limestone, with a precision of ± 0.31 ‰, and corrected for the C atom added during derivatization.

Sediment horizons between 0 and 10 cm preserved in formalin were sieved over a 300µm mesh. Macrofauna were extracted under a binocular microscope, identified to broad taxonomic level, air dried in pre-weighed tin capsules, and weighed. In some cases multiple individuals were pooled to create samples large enough for analysis. Fauna were de-carbonated by dropwise addition of 0.1M HCl, followed by air drying at 50°C. Calcareous foraminifera and bivalves which were too small for manual removal of shells were de-carbonated with 6N HCl. Fauna were analysed for their C contents and isotopic signature using a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a Delta^{Plus} XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen). Carbon contents was quantified using the area under the mass spectrometer response curve, against National Institute of Standards and Technology reference material 1547 peach leaves (repeat analysis gave precision ± 0.35 %). Isotopic data were traceable to IAEA reference materials USGS40 and USGS41 (both L-glutamic acid), with a precision ± 0.13 ‰.

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2.4 Data treatment

Respiration of added algal C was calculated for cores subjected to the algae treatment. The amount of excess DI^{13}C in each sample was calculated by first subtracting the natural abundance of ^{13}C in DIC. This was scaled up to give the total amount of DIC from the added algae at each sample timepoint, and corrected for water removed and added during sampling. Respiration rate was calculated for each core by placing a line of best fit through the amount of added ^{13}C over time, and normalised to surface area.

Bacterial incorporation of ^{13}C was calculated by first subtracting the natural abundance of ^{13}C from the isotopic signature of each PLFA (data published in Bell et al., 2017), where the difference exceeded the precision of the analytical technique, to give the amount of added C in each compound. Bacterial incorporation was then

calculated using the 4 bacteria-specific PLFAs isoC14:0, isoC15:0, antisoC15:0, and isoC16:0, following Boschker and Middelburg (2002). Uptake of ^{13}C into these bacteria-specific PLFAs was summed, and scaled up on the basis that they together account for 14% of total bacterial PLFA, and that PLFAs account for 5.6% of total bacterial biomass. For samples in the bicarbonate treatment further scaling up was applied, to account for the fact that the addition of ^{13}C bicarbonate was calculated to result in a porewater DIC pool that was 22 atom % ^{13}C.

Faunal uptake of added ^{13}C was calculated by subtracting ^{13}C attributable to its natural abundance in the appropriate taxon (data published in Bell et al., 2017 a) from faunal isotopic signatures, where the difference exceeded the precision of the analytical technique, and multiplying by the quantity of organic C in each specimen. Specimens were summed for each core, and the value multiplied by 2, to account for only half of each horizon being used for faunal extraction.

3. Results

Data files can be accessed at DOIxxxx.

3.1 3.1 Respiration

Respiration rates measured in algae addition experiments varied from 0.03 mg C m^{-2} h^{-1} at the off vent site to 0.15 mg C m^{-2} h^{-1} at Middle Sister (Fig. 2).

3.2 3.2 Bacterial uptake and PLFA suite

In the algae addition experiments, total bacterial uptake of C throughout the experiment was maximal at Middle Sister and Hook Ridge (1.30-1.91 and 1.25 mg C m^{-2}, respectively), and minimal at the off vent site (0.25-0.77 mg C m^{-2}, Fig. 3). In bicarbonate addition experiments, in which incorporation of ^{13}C into bacterial PLFAs represents chemosynthesis, bacterial incorporation of bicarbonate was maximal at the off vent site (0.05-0.10 mg C m^{-2}), and was also detectable in one of the replicates at Middle Sister (0.003 mg C m^{-2}, close to detection limits, so this value is treated with caution), however it was not detectable at Hook Ridge.

The PLFA suites at all sites were qualitatively similar. They were dominated by C16:0, C16:1ω7c, and C18:1ω7, which together constituted 42 ± 2% of total PLFAs (Fig. 4). This is at the high end of contributions from these compounds elsewhere, such as 34-45% in the Arabian Sea, and 41% on the Galicia Bank (Kunihiro et al., 2014). The relatively high proportions of C16:1ω7 and C18:1ω7 are indicative of the presence of chemosynthetic and specifically sulphide oxidising bacteria (Colaco et al., 2007). In addition C18:1ω9, which is

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linked to endosymbionts in vent mussels, and C18:1ω13, which is associated with methylotrophic bacteria were also present (Colaco et al., 2007).

In both algae and bicarbonate addition experiments, ^{13}C incorporation into PLFAs was dominated by C16:0, followed by C18:1ω9 and the sulphide oxidiser indicators C16:1ω7 and C18:1ω7 (Fig 4).

3.3 3.3 Faunal uptake

Faunal uptake of added C differed between A and B replicate cores in all experiments except the algae addition at the off vent site, and bicarbonate addition at Middle Sister (Fig. 5).

In algae addition experiments faunal uptake was similar between the off vent site and one of the Hook Ridge cores (~0.03 mg C m^{-2}), while the other Hook Ridge core showed very low faunal C uptake. Considerably greater faunal uptake (0.12 mg C m^{-2}) was observed in one of the replicate cores from Middle Sister (Fig. 5).

In bicarbonate addition experiments, measurable uptake of ^{13}C by fauna was observed at all sites. It was maximal at Hook Ridge (0.02 mg C m^{-2} in one replicate), and the off vent and Middle Sister sites showed similar values (Table 2, Fig. 5).

Small size of individuals meant that organisms had to be pooled for isotopic analysis, limiting the taxonomic resolution of the faunal uptake data. Although limited in this way, the data show that faunal uptake of ^{13}C in both algae and bicarbonate addition experiments was mostly carried out by either polychaetes, or 'mixed macrofauna' (Fig. 6). This latter category contained variously bivalves, crustaceans, echinoderms, nematodes and foraminifera, in cases where those groups were not present in sufficient numbers for separate reporting of their C uptake. When a group was present in sufficient quantity it was analysed separately. As with total macrofaunal ^{13}C uptake, there was considerable variability between replicate cores in the most abundant taxonomic groups. In addition, meiofaunal organisms took up ^{13}C at Middle Sister, and the bicarbonate ^{13}C that was transferred to macrofauna at Hook Ridge was mostly observed in amphipod crustaceans.

4. Discussion

4.1 Occurrence of inorganic C fixation

The results of bicarbonate addition experiments show evidence for occurrence of benthic C-fixation at all sites, and transfer of that C to the macrofauna, in the form of isotopic enrichment of bacterial PLFAs at the off-vent and Middle Sister sites (Fig. 3), and of macrofauna at the Hook Ridge and Middle Sister sites (Fig. 5). The

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quantities of bicarbonate ^{13}C detected in bacterial and faunal biomass were low, and tended to be 1 to 2 orders of magnitude smaller than equivalent values for algae addition experiments (Table 2). We have confidence that the values reported are above detection limits, in that data were only used where the enrichment of organisms or PLFAs above their natural background signatures was greater than the analytical precision of the method. The greatest quantities of bacterial uptake were measured at the off-vent site (Fig. 3), and the greatest quantity transferred to the fauna was measured at Hook Ridge (Fig. 5), however, due to the low values measured and the evident patchiness of faunal communities we do not feel these differences are suitable for further discussion.

The most striking result of the bicarbonate addition experiments was that evidence for benthic C fixation was found at all sites, not only at the hydrothermally influenced Hook Ridge. Further, the site showing the largest amount of incorporation of bicarbonate ^{13}C into bacterial PLFAs was the off-vent 'control' site (Table 2, Fig. 3). This is consistent with the occurrence of siboglinids at all sites. These host chemosynthetic endosymbionts most of which conduct sulphide oxidation (Thornhill et al., 2008; Georgieva et al., 2015). It should be noted that the evidence for inorganic C fixation comes from PLFAs in the bulk sediment, while isotopic signatures of siboglinids did not show enrichment above background values. Therefore the occurrence of benthic C fixation is not only associated with siboglinids.

Experiments were designed to replicate natural conditions as far as practically possible, while being limited to shipboard rather than in situ methods. One result of this is that the sediment contained in cores was detached from the upward flux of hydrothermal fluid, and the electron donors it supplied. This could have limited inorganic C fixation, which would have impacted the rates measured at Hook Ridge. We suggest however that this is not a serious limitation, as Hook Ridge was rather mildly hydrothermal. Vent endemic fauna were almost absent (Bell et al., 2016), there was no increase in faunal biomass close to venting, downcore profiles of alkalinity, nitrate and ammonium were consistent with normal microbial processes, and hydrothermal advection rates were 9-33 cm y^{-1} (Aquilina et al., 2013). At these low advection rates we suggest that there would not have been sufficient time during our ~60 h experiments for a noticeable depletion in availability of electron donors supplied by hydrothermal fluid.

The evidence suggests that while the amount of benthic C-fixation was always low, it was not restricted to environments typically thought of as chemosynthetic (sedimented or hard substrate hydrothermal vents, methane seeps, or organic falls (Bernardino et al., 2012)). Thus, benthic C-fixation appears to play a role in benthic C-cycling at a much wider range of sites and over a much larger area of the seafloor than previously thought. This is supported by linear inverse modelling of C-cycling at the sites in this study, which led Bell et al. (2017b) to

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suggest that chemosynthetic support for ecosystems may have a far greater spatial extent than previously thought, extending beyond those which are directly hydrothermally influenced. Similar results have also been reported in non-hydrothermal, but methane rich sediments on the South Georgia margin, where assimilation of ^{13}C labelled bicarbonate into bacterial biomass, and transfer into macrofauna was also observed (Would et al., 2019). In addition, in situ observations of benthic C fixation have also been made at mesotrophic, abyssal sites in the eastern equatorial Pacific, which were not associated with hydrothermal or methane seep activity (Sweetman et al. 2018). In that study incorporation of ^{13}C labelled bicarbonate into bacterial PLFAs was observed at 2 sites separated by 100's of kilometres, at rates similar to bacterial assimilation of phytodetritus C at the same sites. Together with global scale modelling completed by Middelburg (2011), these studies suggest that chemoautotrophic C fixation may be considerably more widespread than previously thought. It is therefore deserving of further study so that it can be quantitatively incorporated into our understanding of the marine C-cycle.

In their study using linear inverse modelling of the benthic food web and C cycle, based on natural isotopic and biomass data, Bell et al. (2017b) modelled a rate for chemosynthesis of 5.76-8.4 mg C m^{-2} d^{-1} at Hook Ridge, and <0.006 mg C m^{-2} d^{-1} at the off-vent site. These modelled rates at Hook Ridge are considerably higher than Hook Ridge benthic C-fixation measured in this study, for which there was evidence (labelled PLFAs), but a rate could not be calculated. The higher modelled rates by Bell et al. (2017 b) may be explained by the fact that a temperature of 50°C was used for the Hook Ridge site, based on previously published conditions of the site (Klinkhammer et al., 2001). Unfortunately, equipment was not available while at sea for measurement of sediment temperature at the study sites, therefore all experiments, including that at Hook Ridge, were conducted at measured bottom water temperatures of 0-1°C. It is likely that the rates measured here for chemosynthetic incorporation of labelled bicarbonate are lower than those that would have been measured in situ. It is also probable that measurable rates could have been detected at Hook Ridge had more samples been available for replicate analyses.

The maximal rate of benthic C-fixation measured in this study was 0.050 mg C m^{-2} d^{-1} , which occurred in one core at the off-vent site. This remains considerably lower than the 0.24-1.02 mg C m^{-2} d^{-1} measured by Molari et al. (2013, rates calculated in Sweetman et al., 2018) at depths ranging between 1207-4381 m on the Iberian margin and in the Mediterranean, and the 1.29 mg C m^{-2} d^{-1} measured by Sweetman et al. (2018) at ~4100 m depth in the Clarion Clipperton Zone. The Bransfield Strait sites in this study were shallower, had higher concentrations of sedimentary organic C, and slightly lower bottom water temperatures than either of the

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previous studies cited. The very low temperatures at which experiments were conducted (1°C at Hook Ridge and 0°C at the off vent site) is likely to have contributed to the slow measured rates of benthic C-fixation. Another factor which may influence benthic C-fixation is the annual flux of photosynthetic C from the surface (Molari et al., 2013; Bell et al., 2017a). The annual flux of POC to the sediments in the Bransfield Strait is greater than in the Clarion Clipperton Zone, and probably than in the Mediterranean as well (Masque et al., 2002; Sweetman et al., 2017), and this may be an additional driver behind the low benthic C-fixation rates observed. Archaeal abundance has been shown to correlate with dark C-fixation, and addition of labile organic material has been shown to increase inorganic C fixation rates, perhaps through a combination of heterotrophy and mixotrophy (Molari et al., 2013). Overall, the factors governing benthic C-fixation rates require investigation. In addition, the pathways (i.e. autotrophic C fixation versus anapleurotic C fixation by heterotrophs, Wegener et al., 2012), energy sources (e.g. sulphide, methane) and organisms responsible for benthic inorganic C fixation have not been identified, and warrant further study.

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4.2 Carbon uptake by macrofauna

Uptake of added C by fauna in isotope tracer experiments usually shows a degree of spatial patchiness (e.g. Woulds et al., 2007), but this seems to have been particularly marked in the Bransfield Strait, mainly at those sites with hydrothermal influence. This is consistent with the patchiness of *Sclerolinum contortum* in replicate cores at Hook Ridge (Bell et al. 2016a). At both Hook Ridge and Middle Sister there was a very marked difference in faunal uptake of algal C between the A and B replicate cores in algae addition experiments (Fig. 5), and this was considerably greater than that observed, for example, in experiments on the Pakistan margin Woulds et al. (2007). This is likely to be due to difference in the biomass of fauna present in each core, and such marked small scale patchiness in faunal communities has been noted previously as a particular feature of SHVs (Levin et al., 2009; Bernardino et al., 2012). Fine scale distribution of fauna is related to variations in concentrations of substrates such as sulphide and methane (Levin et al., 2003), therefore the patchiness observed especially at Hook Ridge is likely related to spatial and temporal fluctuation in hydrothermal advection.

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Faunal uptake of added C appeared to be greatest at Middle Sister in algae addition experiments, and at Hook Ridge in bicarbonate addition experiments, however the variation between replicate cores limits conclusions that can be drawn. Previous isotope tracing experiments have noted correlations between biomass of taxa and the amount of C they take up (e.g. Woulds et al., 2007). Further, there was no systematic variation in biomass-specific C uptake (0.026-0.13 ug C uptake / mg C biomass) between sites, therefore the patterns observed here in faunal C uptake are likely to result from variation in biomass present in each experimental core.

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Similarly, the identities of fauna responsible for ^{13}C uptake was variable between replicate cores (Fig. 6), and this is also likely to have been driven by variation in the macrofaunal community present in each core. The prevalence and variable importance of the 'mixed macrofauna' category indicates that in some cases a fairly diverse assemblage was engaged in C uptake and processing.

Previous studies have suggested that SHVs tend to exhibit relatively high biomass macrofaunal communities, sustained by the additional food source provided by chemosynthesis (Bernadino et al., 2012), and this leads to an expectation that the macrofauna may be particularly active in processing of organic C in the sediment, in line with other food rich environments such as estuaries and fjords (Moodley et al., 2000; 2005; Witte et al., 2003a). This was not the case in the algae addition experiments, with faunal uptake accounting for only 0.05-2.2 % of total biological ^{13}C processing (Fig. 7). This is similar to the role of faunal C uptake in overall C processing seen at deep, organic carbon poor sites such as at 2170 m depth off NW Spain (2.2 %, Moodley et al., 2002), or at 1552 m depth in the Eastern Mediterranean (0.2 %, Moodley et al., 2005), and is lower than that at 4800 m depth on the Porcupine Abyssal Plain (1.5-26 %, Witte et al., 2003b). Such sites tend to have lower OC concentrations and lower macrofaunal biomass (Woulds et al., 2016) than was observed in the Bransfield Strait, therefore the unusually small role of macrofaunal in C uptake in the Bransfield Strait may be due to low temperatures. Both low temperature and food scarcity have previously been observed to limit metabolic rates in polar environments (Brockington and Peck, 2001; Sommer and Portner, 2002). Another possible explanation for the rather small amount of macrofaunal C uptake at the Hook Ridge site may be that the macrofaunal community, which was composed almost entirely of non vent-obligate, ambient Southern Ocean taxa (Bell et al., 2016a), had reduced levels of function due to the stress imposed by living at a site influenced by hydrothermal fluid. The toxicity and relatively high temperature of their environment (compared to non-hydrothermal Southern Ocean benthic settings) may have resulted in reduced C uptake activity. Therefore, macrofaunal biomass and C processing activity were limited by a hydrothermal flux that was sufficient to limit functioning and preclude occurrence of some some locally common taxa, but insufficient to sustain a high biomass, vent endemic macrofaunal community as seen in other SHVs (Bell et al., 2016 a).

Siboglinid polychaetes, known to host chemosynthetic endosymbionts, were present at all study sites (Bell et al., 2016 a), but were not found to make a substantial contribution to uptake of added ^{13}C. This is to be expected in the algae addition experiments, as siboglinids would have direct access to algal C (except possibly via DOC). Most specimens recovered from biocarbonate addition experiments also showed $\delta^{13}C$ values indistinguishable

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from their natural signature, with one exception at the Middle Sister site which was enriched compared to the natural signature by 3.2 ‰. The fact that siboglinids did not have a major role in C fixation and cycling in our experiments may have been partly due to their low abundances in experiment cores compared to patches where they were maximally abundant (Bell et al., 2016a), or because experiments were not long enough for uptake by endosymbionts. Nonetheless, our findings show a much reduced role for siboglinids compared to suggestions made in previous publications. Aquilina et al. (2014) suggested that *Siboglinum sp.* at Hook Ridge may be sufficiently abundant to be conduits for a quantitatively meaningful flux of dissolved iron out of the sediment, and Bell et al. (2017 b) found that they may be a key taxon facilitating input of chemosynthetic C into the food web. In agreement with the point made by Bell et al. (2016a), the spatial distribution of siboglinids is extremely patchy, and thus their role in benthic biogeochemical processes is spatially heterogeneous (Bell et al., 2017a, b).

4.3 Carbon processing and SHVs as biogeochemical hotspots

Respiration rates measured in the algae addition experiments were maximal at Middle Sister, and minimal at the off-vent site (Fig. 2). Temperature is often recognised as a dominant control on benthic respiration rates (e.g. Moodley et al., 2005; Woulds et al., 2009), however these experiments were all conducted within 1°C of each other, so temperature is unlikely to have driven differences in respiration rates. Instead, the differences between sites may have been driven by differences in bacterial biomass (Table 1), which was maximal at Middle Sister and minimal at the off-vent site. The bacteria are often found to account for a large majority of benthic community biomass, and are thus usually assumed to be responsible for the majority of benthic community respiration (e.g. Heip et al., 2001). The measured respiration rates were similar to those measured at 2170 m on the NW margin of Spain (Moodley et al., 2002), and on the Porcupine Abyssal Plain (Witte et al., 2003b), both of which were considerably deeper, and had lower sediment organic C concentrations, but higher bacteria biomass (Woulds et al., 2016). They were also lower than respiration rates measured at similar depths in the Eastern Mediterranean (Moodley et al., 2005), and Arabian Sea (Woulds et al., 2009). These sites showed similar bacteria biomass to the Bransfield Strait, but were all considerably warmer (7-14°C, Woulds et al., 2016), therefore the low ambient temperatures of the Southern Ocean appeared to reduce respiration rates.

It has been suggested that reducing benthic environments are often hotspots of faunal biomass and biogeochemical cycling due to the increased availability of labile food sources supplied by chemosynthesis (Bernardino et al., 2012). In this study, the hydrothermally active site Hook Ridge showed rates of respiration and bacterial uptake of algal C that were intermediate between the two non-hydrothermally active sites (Figs. 2, 3). Whilst comparison between sites is limited by very marked faunal patchiness, the amount of faunal uptake of

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algal ^{13}C at Hook Ridge was similar to that at the off-vent control site, while that at Middle Sister was, in one replicate, considerably greater (Fig. 5). This suggests that SHVs are not necessarily biogeochemical cycling hotspots, as in algae addition experiments the overall amount of added C processed by the benthic community was not greater than that observed at non-hydrothermal sites (Fig. 8). In line with this, biological processing of added C in the algae addition experiments did not show a major role for faunal C uptake as we hypothesised, but was instead dominated by respiration, as is typically observed at relatively deep, cold sites (Woulds et al., 2009). The Middle Sister site showed the greatest amount of biological processing of added algal C, which was probably attributable to it having the greatest bacterial biomass and organic carbon concentrations, and the fact that the macrofaunal community, composed mostly of ambient Southern Ocean taxa, will have been functioning without the stress imposed by hydrothermal fluid.

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5. Conclusions

The main fate of photosynthetic C was respiration in common with other deeper and more food limited sites. The rates of respiration and C uptake by both macrofaunal and bacteria that we measured were comparatively low, and this is attributable to the low temperature of the experiments, and the toxicity and thermal stress caused by hydrothermal fluid. The hydrothermal site (Hook Ridge) in this study did not show more rapid C-cycling than other similar experiments as we hypothesised it would. Benthic fixation of inorganic was observed at all sites, and quantified at 2 out of 3 sites. While the rates were low compared to other similar experiments, the fact that the greatest amount of benthic C fixation occurred at the off vent site suggests that benthic C fixation may not be restricted to hydrothermal and other reducing settings. We suggest that it could be an important aspect of the marine C-cycle, and warrants further study.

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Data Availability

Data sets can be found at DOIxxxx

Author Contributions

Experiments were conducted by C. Woulds and A. Glover. All authors contributed to analysis of samples, and commented on the manuscript.

Acknowledgements

This work was funded by Antarctic Science Ltd., and NERC (grant NE/J013307/1). The authors would like to thank Prof. Paul Tyler, as well as the officers and crew of RRS James Cook, and the on-board scientific party on cruise JC 55. We would also like to thank Elisa Neame for assistance with extracting macrofauna.

References

Aquilina, A., Connelly, D. P., Copley, J. T., Green, D. R. H., Hawkes, J. A., Hepburn, L. E., Huvenne, V. A. I., Marsh, L., Mills, R. A., and Tyler, P. A.: Geochemical and Visual Indicators of Hydrothermal Fluid Flow through a Sediment-Hosted Volcanic Ridge in the Central Bransfield Basin (Antarctica), Plos One, 8, 2013.

Aquilina, A., Homoky, W. B., Hawkes, J. A., Lyons, T. W., and Mills, R. A.: Hydrothermal sediments are a source of water column Fe and Mn in the Bransfield Strait, Antarctica, Geochimica Et Cosmochimica Acta, 137, 64-80, 2014.

Bell, J. B., Aquilina, A., Woulds, C., Glover, A. G., Little, C. T. S., Reid, W. D. K., Hepburn, L. E., Newton, J., and Mills, R. A.: Geochemistry, faunal composition and trophic structure in reducing sediments on the southwest South Georgia margin, Royal Society Open Science, 3, 2016b.

Bell, J. B., Reid, W. D. K., Pearce, D. A., Glover, A. G., Sweeting, C. J., Newton, J., and Woulds, C.: Hydrothermal activity lowers trophic diversity in Antarctic hydrothermal sediments, Biogeosciences, 14, 5705-5725, 2017a.

Bell, J. B., Woulds, C., Brown, L. E., Sweeting, C. J., Reid, W. D. K., Little, C. T. S., and Glover, A. G.: Macrofaunal ecology of sedimented hydrothermal vents in the Bransfield Strait, Antarctica, Frontiers in Marine Science, 3, 2016a.

Bell, J. B., Woulds, C., and Oevelen, D. v.: Hydrothermal activity, functional diversity and chemoautotrophy are major drivers of seafloor carbon cycling, Scientific Reports, 7, 12025, 2017b.

Bernardino, A. F., Levin, L. A., Thurber, A. R., and Smith, C. R.: Comparative Composition, Diversity and Trophic Ecology of Sediment Macrofauna at Vents, Seeps and Organic Falls, Plos One, 7, 2012.

Boschker, H. T. S. and Middelburg, J. J.: Stable isotopes and biomarkers in microbial ecology, FEMS Microbiology Ecology, 40, 85-95, 2002.

Brockington, S., Peck, L. S.: Seasonality of respiration and ammonium excretion in the Antarctic echinoid Sterechinus neumayeri, Marine Ecology Progress Series, 219, 159-168, 2001.

Colaco, A., Desbruyeres, D., and Guezennec, J.: Polar lipid fatty acids as indicators of trophic associations in a deep-sea vent system community, Marine Ecology-an Evolutionary Perspective, 28, 15-24, 2007.

Georgieva, M. N., Wicklund, H., Bell, J. B., Eilertsen, M. H., Mills, R. A., Little, C. T. S., Glover, A. G.: A chemosynthetic weed: the tubeworm *Sclerolinum contortum* is a bipolar, cosmopolitan species, BMC Evolutionary Biology, 15, article 280, 2015.

Heip, C. H. R., Duineveld, G., Flach, E., Graf, G., Helder, W., Herman, P. M. J., Lavaleye, M., Middelburg, J. J., Pfannkuche, O., Soetaert, K., Soltwedel, T., de Stigter, H., Thomsen, L., Vanaverbeke, J., and de Wilde, P.: The role of the benthic biota in sedimentary metabolism and sediment-water exchange processes in the Goban Spur area (NE Atlantic), Deep Sea Research Part II, 48, 3223-3243, 2001.

Klinkhammer, G. P., Chin, C. S., Keller, R. A., Dahlmann, A., Sahling, H., Sarthou, G., Petersen, S., and Smith, F.: Discovery of new hydrothermal vent sites in Bransfield Strait, Antarctica, Earth and Planetary Science Letters, 193, 395-407, 2001.

Kunihiro, T., Veuger, B., Vasquez-Cardenas, D., Pozzato, L., Le Guitton, M., Moriya, K., Kuwae, M., Omori, K., Boschker, H. T. S., and van Oevelen, D.: Phospholipid-Derived Fatty Acids and Quinones as Markers for Bacterial Biomass and Community Structure in Marine Sediments, Plos One, 9, 2014.

Levin, L. A., Mendoza, G. F., Konotchick, T., and Lee, R.: Macrobenthos community structure and trophic relationships within active and inactive Pacific hydrothermal sediments, Deep-Sea Research Part Ii-Topical Studies in Oceanography, 56, 1632-1648, 2009.

Levin, L. A., Ziebis, W., Mendoza, G. F., Growney, V. A., Tryon, M. D., Brown, K. M., Mahn, C., Gieskes, J. M., Rathburn, A. E.: Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulphide concentration and fluid flow, Marine Ecology Progress Series, 265, 123-139, 2003.

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Main, C. E., Ruhl, H. A., Jones, D. O. B., Yool, A., Thornton, B., and Mayor, D. J.: Hydrocarbon contamination affects deep-sea benthic oxygen uptake and microbial community composition, Deep-Sea Research Part I-Oceanographic Research Papers, 100, 79-87, 2015.

Masque, P., Isla, E., Sanchez-Cabeza, J. A., Palanques, A., Bruach, J. M., Puig, P., and Guillen, J.: Sediment accumulation rates and carbon fluxes to bottom sediments at the Western Bransfield Strait (Antarctica), Deep-Sea Research Part Ii-Topical Studies in Oceanography, 49, 921-933, 2002.

Middelburg, J. J.: Chemoautotrophy in the ocean, Geophysical Research Letters, 38, 2011.

Molari, M., Manini, E., and Dell'Anno, A.: Dark inorganic carbon fixation sustains the functioning of benthic deep-sea ecosystems, Global Biogeochemical Cycles, 27, 212-221, 2013.

Moodley, L., Boschker, H. T. S., Middelburg, J. J., Pel, R., Herman, P. M. J., de Deckere, E., and Heip, C. H. R.: Ecological significance of benthic foraminifera: ^{13}C labelling experiments, Marine Ecology Progress Series, 202, 289-295, 2000.

Moodley, L., Middelburg, J. J., Boschker, H. T. S., Duineveld, G. C. A., Pel, R., Herman, P. M., and Heip, C. H. R.: Bacteria and foraminifera: Key players in a short-term deep-sea benthic response to phytodetritus, Marine Ecology Progress Series, 236, 23-29, 2002.

Moodley, L., Middelburg, J. J., Soetaert, K., Boschker, H. T. S., Herman, P. M., and Heip, C. H. R.: Similar rapid response to phytodetritus deposition on shallow and deep-sea sediments, Journal of Marine Research, 63, 457-469, 2005.

Portail, M., Olu, K., Dubois, S. F., Escobar-Briones, E., Gelinas, Y., Menot, L., and Sarrazin, J.: Food-Web Complexity in Guaymas Basin Hydrothermal Vents and Cold Seeps, Plos One, 11, 2016.

Sahling, H., Wallmann, K., Dahlmann, A., Schmaljohann, R., and Petersen, S.: The physicochemical habitat of Sclerolinum sp at Hook Ridge hydrothernial vent, Bransfield Strait, Antarctica, Limnology and Oceanography, 50, 598-606, 2005.

Sommer, A. M., Portner, H. O.: Metabolic cold adaptation in the lugworm Arenicola marina: comparison of a North Sea and a White Sea population, Marine Ecology Progress Series, 240, 171-182, 2002.

Soto, L. A.: Stable carbon and nitrogen isotopic signatures of fauna associated with the deep-sea hydrothermal vent system of Guaymas Basin, Gulf of California, Deep-Sea Research Part Ii-Topical Studies in Oceanography, 56, 1675-1682, 2009.

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Sweetman, A. K., Levin, L. A., Rapp, H. T., and Schander, C.: Faunal trophic structure at hydrothermal vents on the southern Mohn's Ridge, Arctic Ocean, Marine Ecology Progress Series, 473, 115-+, 2013.

Sweetman, A. K., Smith, C. R., Shulse, C. N., Maillot, B., Lindh, M., Church, M. J., Meyer, K., Oevelen, D. v., Stratmann, T., and Gooday, A. J.: Key role of bacteria in the short-term cycling of carbon at the abyssal seafloor, Limnology and Oceanography, 9999, 1-20, 2018.

Sweetman, A. K., Thurber, A. R., Smith, C. R., Levin, L. A., Mora, C., Wei, C. L., Gooday, A. J., Jones, D. O. B., Rex, M. A., Yasuhara, M., Ingels, J., Ruhl, H. A., Frieder, C. A., Danovaro, R., Wurzberg, L., Baco, A. R., Grupe, B. M., Pasulka, A., Meyer, K. S., Dunlop, K. M., Henry, L.-A., and Roberts, M.: Major impacts of climate change on deep-sea benthic ecosystems, Elementa: Science of the Anthropocene, 5, 2017.

Thornhill, D. J., Wiley, A. A., Campbell, A. L., Bartol, F. F., Teske, A., Halanych, K. M.: Endosymbionts of *Siboglinum fjordicum* and the phylogeny of bacterial endosymbionts in siboglinidae (Annelida), Biological Bulletin, 214, 135-144, 2008.

van Oevelen, D., Bergmann, M., Soetaert, K., Bauerfeind, E., Hasemann, C., Klages, M., Schewe, I., Soltwedel, T., and Budaeva, N. E.: Carbon flows in the benthic food web at the deep-sea observatory HAUSGARTEN (Fram Strait), Deep-Sea Research Part I-Oceanographic Research Papers, 58, 1069-1083, 2011.

van Oevelen, D., Soetaert, K., and Heip, C.: Carbon flows in the benthic food web of the Porcupine Abyssal Plain: The (un)importance of labile detritus in supporting microbial and faunal carbon demands, Limnology and Oceanography, 57, 645-664, 2012.

Wegener, G., Bausch, M., Holler, T., Thang, N. M., Mollar, X. P., Kellermann, M. Y., Hinrichs, K. U., and Boetius, A.: Assessing sub-seafloor microbial activity by combined stable isotope probing with deuterated water and 13C-bicarbonate, Environmental Microbiology, 14, 1517-1527, 2012.

Witte, U., Aberle, N., Sand, M., and Wenzhofer, F.: Rapid response of a deep-sea benthic community to POM enrichment: an *in situ* experimental study, Marine Ecology Progress Series, 251, 27-36, 2003 a.

Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.-R., Jorgensen, B. B., and Pfannkuche, O.: In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor, Nature, 424, 763-766, 2003 b.

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Woulds, C., Andersson, J. H., Cowie, G. L., Middelburg, J. J., and Levin, L. A.: The short-term fate of organic carbon in marine sediments: Comparing the Pakistan margin to other regions, Deep Sea Research Part II: Topical Studies in Oceanography, 56, 393-402, 2009.

Woulds, C., Bouillon, S., Cowie, G., Drake, E., Middelburg, J. J., and Witte, U.: Patterns of carbon processing at the seafloor: the role of faunal and microbial communities in moderating carbon flows, Biogeosciences, 13, 1-15, 2016.

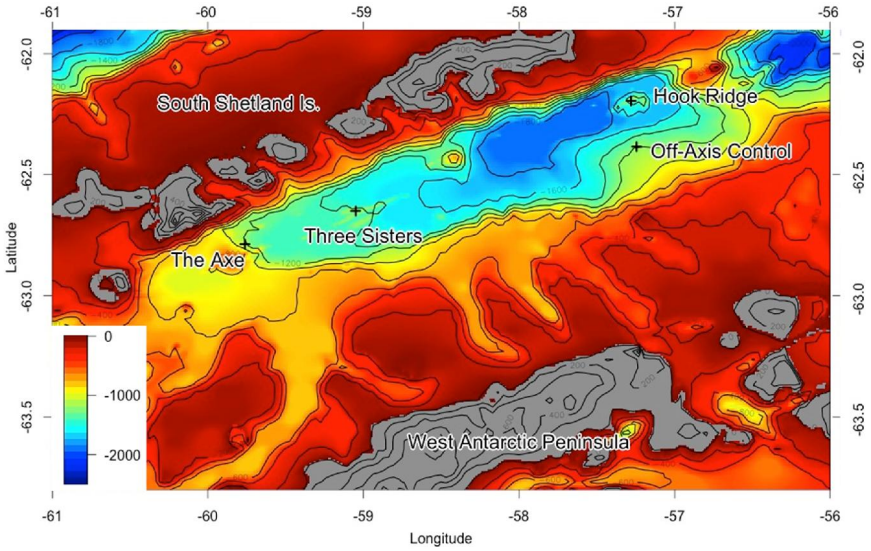
Woulds, C., Cowie, G. L., Levin, L. A., Andersson, J. H., Middelburg, J. J., Vandewiele, S., Lamont, P. A., Larkin, K. E., Gooday, A. J., Schumacher, S., Whitcraft, C., Jeffreys, R. M., and Schwartz, M. C.: Oxygen as a control on seafloor biological communities and their roles in sedimentary carbon cycling, Limnology and Oceanography, 52, 1698-1709, 2007.

Site	Lat.	Long.	Depth (m)	Temperature	Sediment wt%Corg in 0-1 cm horizon	Macrofaunal Biomass (mg C m^{-2})	Bacterial Biomass (mg C m^{-2})
Off-Vent	62.3842 S	57.2440 W	1150	0	1.35	1091	314±145
Hook Ridge	62.1924 S	57.2783 W	1054	1	0.97	318	451±21
Middle Sister	62.6552 S	59.0502 W	1311	0	1.40	374	575±394

Table 1. Site characteristics, all except bacterial biomass are from Bell et al. (2016).

Site	Treatment and Replicate	Amount Respired (mg C m^{-2})	Respiration Rate (mg C m^{-2} h^{-1})	Bacterial Uptake (mg C m^{-2})	Macrofaunal Uptake (mg C m^{-2})
Off-Vent	Algae A	1.23	0.025	0.25	0.027
Off-Vent	Algae B	0.75	0.015	0.77	0.034
Off-Vent	Bicarbonate A	N/A	N/A	0.053	0.0009
Off-Vent	Bicarbonate B	N/A	N/A	0.102	low
Hook Ridge	Algae A	4.97	0.087	n.d.	0.033
Hook Ridge	Algae B	4.06	0.071	1.25	0.003
Hook Ridge	Bicarbonate A	N/A	N/A	n.d.	0.021
Hook Ridge	Bicarbonate B	N/A	N/A	low	low
Middle Sister	Algae A	7.16	0.13	1.91	0.004
Middle Sister	Algae B	8.37	0.15	1.30	0.12
Middle Sister	Bicarbonate A	N/A	N/A	0.00	0.003
Middle Sister	Bicarbonate B	N/A	N/A	0.003*	0.003

Table 2. Amount of C in pools at experiment end, and respiration rates (algae addition experiments only). N/A indicates that it was not appropriate to measure respiration in bicarbonate addition experiments, n.d. indicates no data due to missing sample, and 'low' indicates unmeasurably low value. The value marked * indicates detectable bacterial ^{13}C uptake, but very close to detection limits, so value to be treated with caution.



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1072 **Figure 1. Map of study sites, adapted from Bell et al. 2016 a. The Off Vent site is marked 'Off-Axis Control', and the**

1073 **Middle Sister site is located where 'Three Sisters' is marked. Depths in m.**

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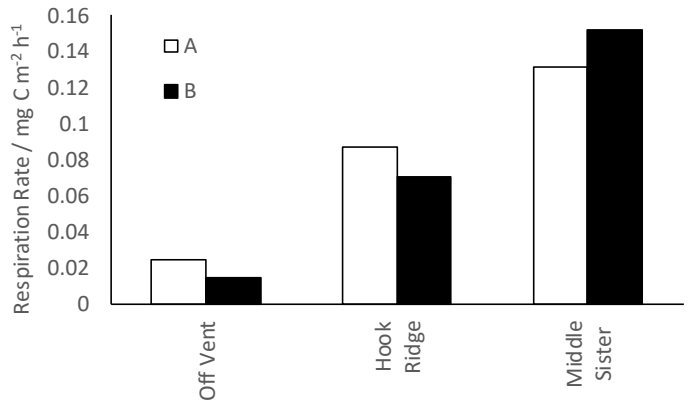

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1078 **Figure 2. Respiration rates measured in algae addition experiments.** A and B refer to the two replicate cores in each

1079 experiment.

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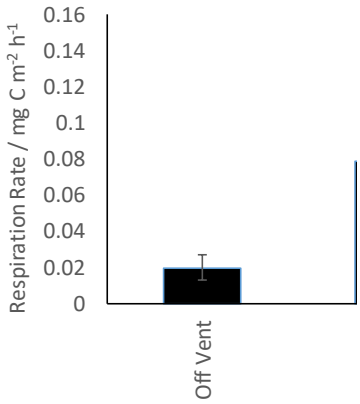

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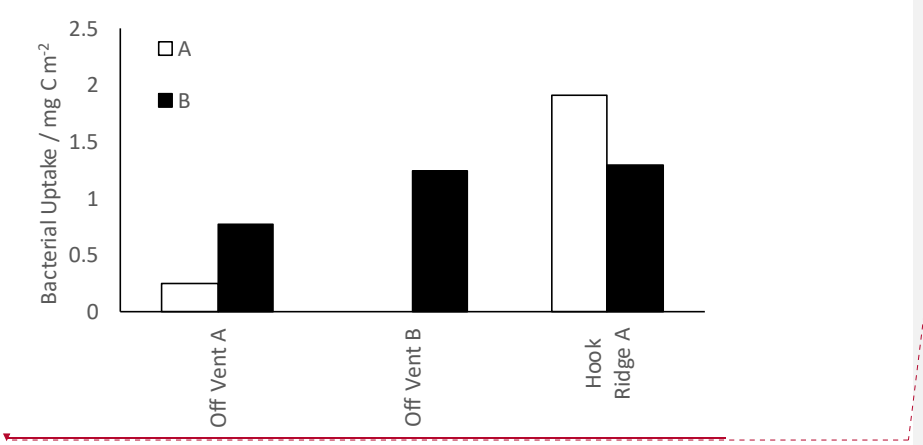

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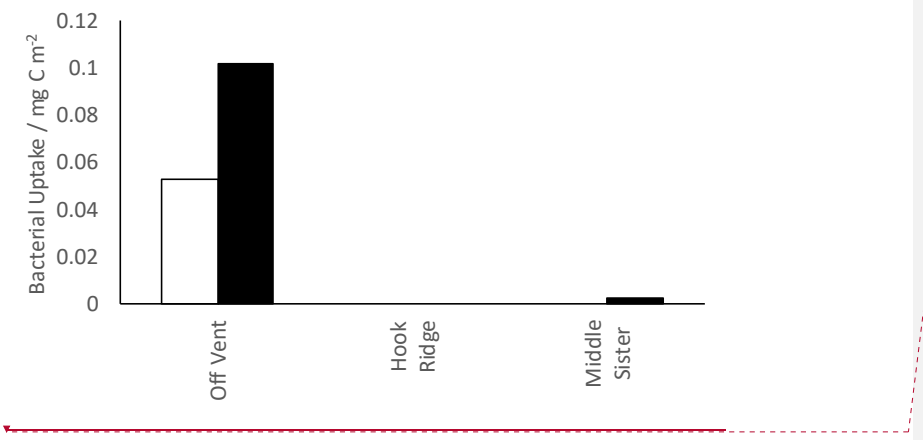

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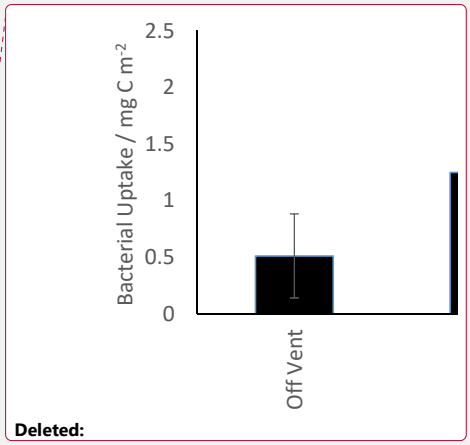
Figure 3. Bacterial uptake measured in A) algae addition experiments; B) bicarbonate addition experiments. Uptake was not quantifiable at Hook Ridge B and Middle Sister A, and sample was not available from Hook Ridge A. A and B refer to the two replicate cores in each experiment.



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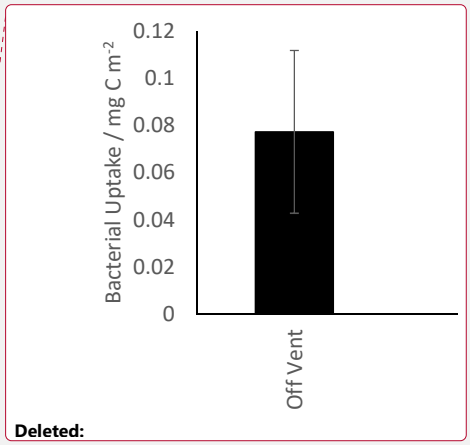

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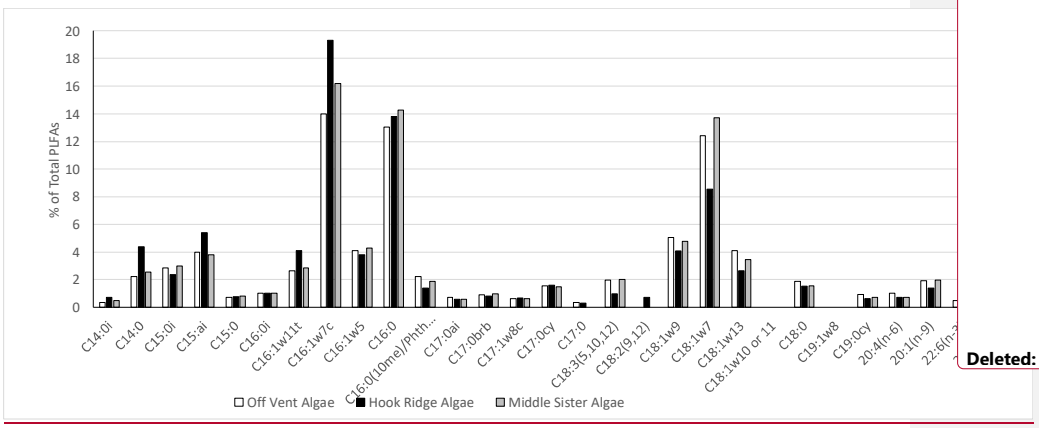

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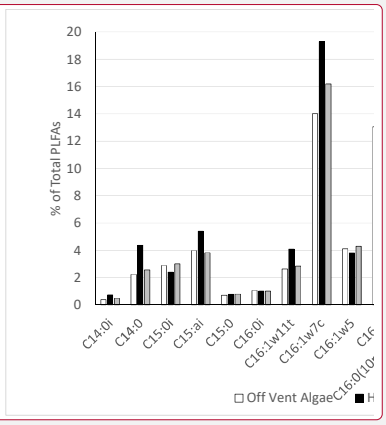

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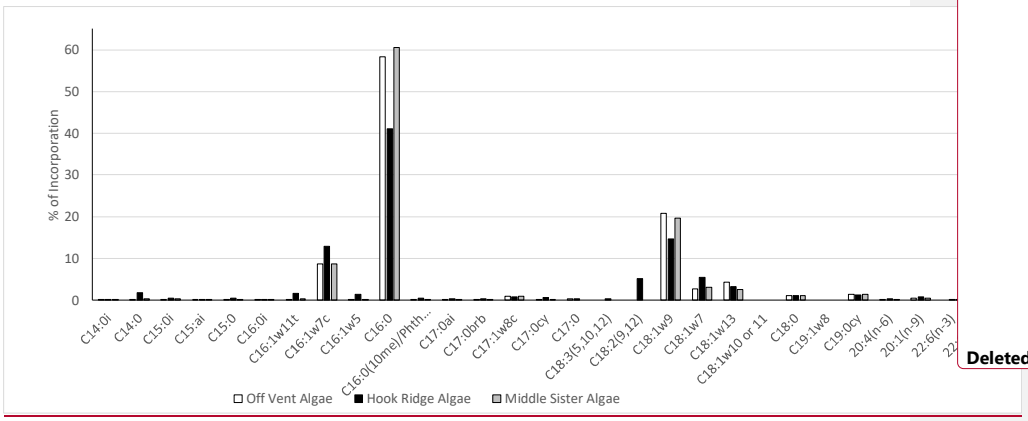

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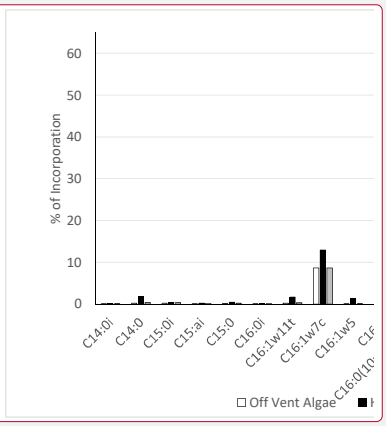

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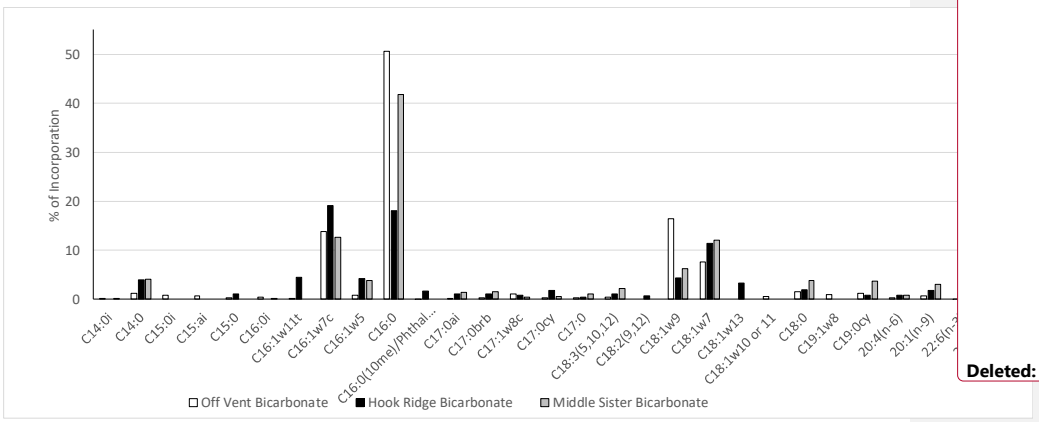

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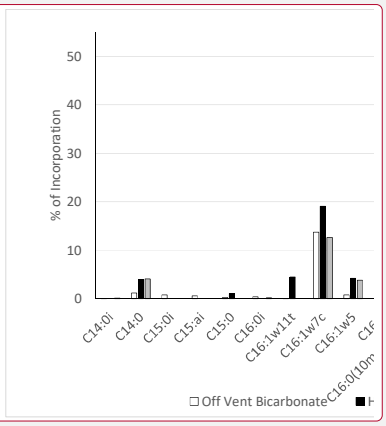

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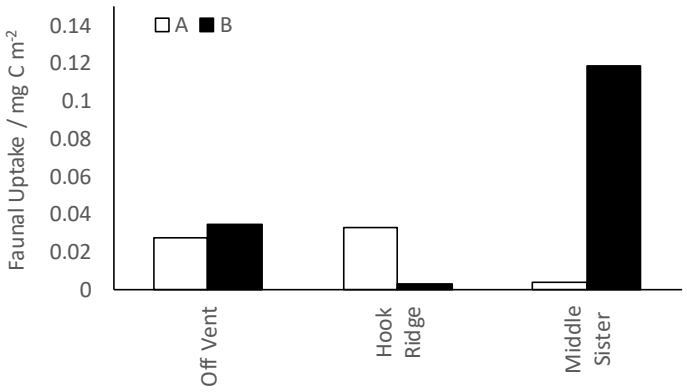
Figure 4. Example PLFA suites – each data series is from one sample, as opposed to being an average across two replicates. A) PLFA suite as % of total PLFAs in algae addition experiments (figure for bicarbonate addition experiments very similar and not shown), B) Composition of ^{13}C uptake into PLFAs in algae addition experiments, and C) Composition of ^{13}C uptake into PLFAs in bicarbonate addition experiments.



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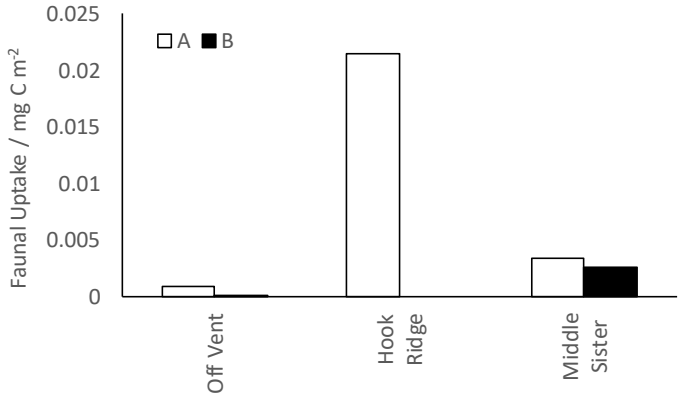
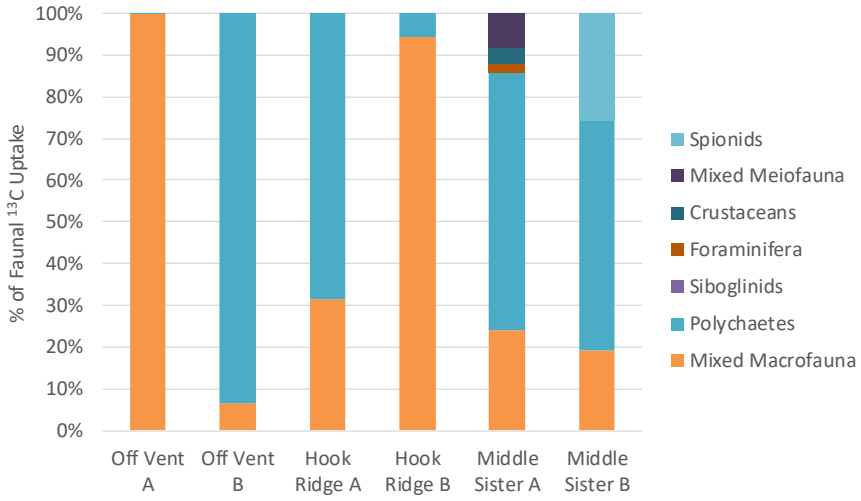


B

Figure 5. Faunal uptake in A) algae addition experiments, and B) bicarbonate addition experiments. A and B refer to the two replicate cores in each experiment.



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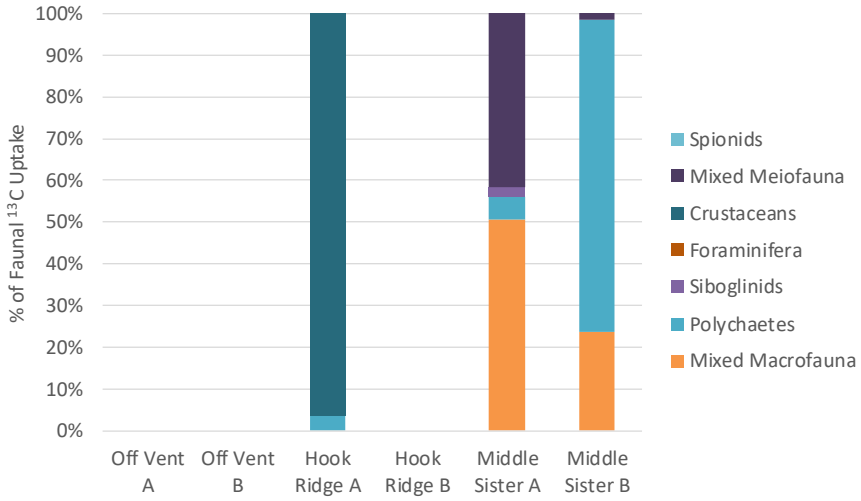
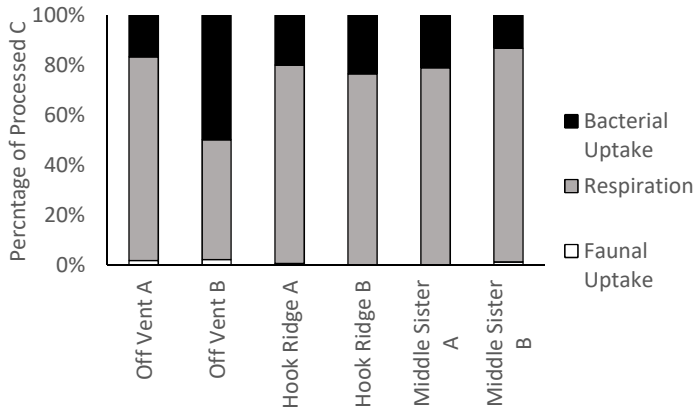


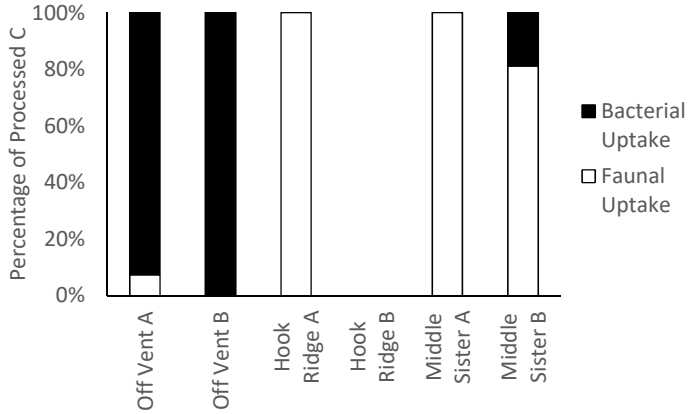
B

Figure 6. Distribution of C uptake amongst taxonomic groups in A) algae addition experiments, and B) bicarbonate addition experiments.



1128

1129 A



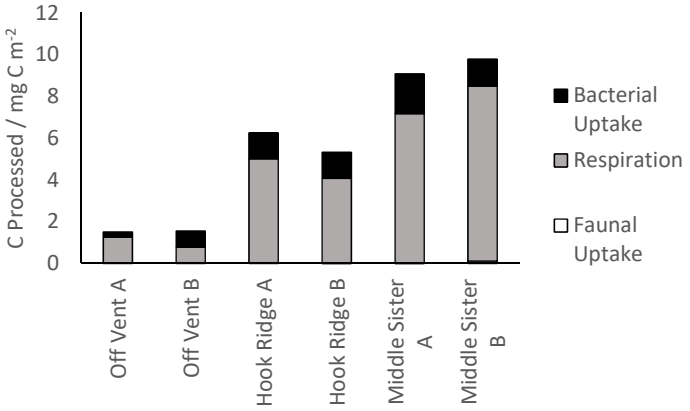
1130

1131 B

1132 **Figure 7. Distribution of biologically processed C between processes for A) algae addition experiments, and B)**
1133 **bicarbonate addition experiments.**

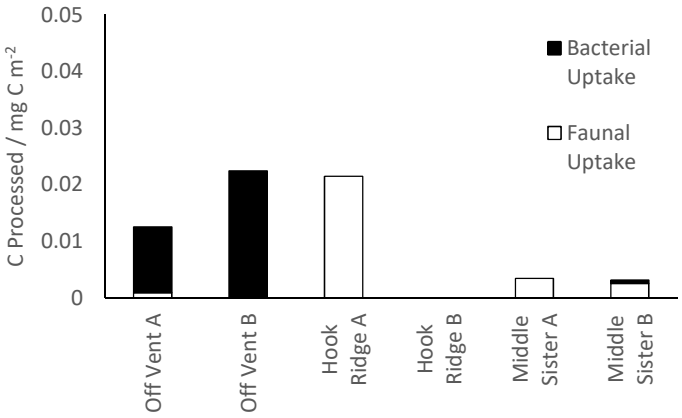
1134

1135



1136

1137 A



1138

1139 B

1140 **Figure 8. Total biological C processing during A) algae addition experiments, B) bicarbonate addition experiments.**

1141